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YEAST FUNGI FROM FRUITS OF MANAUS AND THEIR IMPORTANCE FOR THE HUMAN MYCOPATHOLOGY

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With the Compliments of Chaves Batista

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Introduction

The study of human mycotic infections due to yeast fungi has recently become a much discussed and widely interesting field among the medical mycologists. It is now almost an accepted fact that the increase

+ University of Lucknow, India

in the prevalence of the yeasts in the human system is related to the clinical use of wide range antibiotics for treatment of bacterial diseases. This can be explained by the fact that the antibiotics have a profound effect on the normal bacterial flora of the human system besides the pathogenic ones, whereas the yeasts are known to be resistant to these drugs. It seems natural that the destruction of bacterial flora by the drugs leaves open available areas of the human system as a neutrilite to the organisms resistant to it i.e. yeasts. In continuation with a series of investigations conducted in the IMUR laboratories related with yeast flora of human and animal system as well as the analysis of their feces in this light, under asymptomatic and symptomatic conditions (Batista et al 1957; 1957a; Silveira 1958; Silveira & Correia 1960; Batista & Silveira 1960; Batista & Pereira 1960), a further project was taken up to study the presence and prevalence of yeasts in the common and uncommon edible fruits (their three layers i.e. skin, pulp and seed) and their possible relation with the various pathological conditions produced by these pathogens in the human system. The

present communication is the first concised report of the results obtained in this field while working on various fruits from Manaus, the region of Amazonas in Brazil, in collaboration with the Instituto Nacional de Pesquisas da Amazonia.

Material & Method

Sixty six different kinds of fruits were collected from divergent localities of Manaus and adjoining areas including forests. Certain of the common varieties were purchased from the local markets. All the fruits were transported to the laboratory for experimental work within 10 hours of the collection. The fruits for the studies were selected with meticulous care, majority (80%) of them being round to oblong in shape with smooth outer surface wall, the rest, however, having regular to irregular ridges on their surface. Completely ripe specimens of all the fruits were utilised for the studies.

The specimens were first subjected to a continuous wash by running tap water for 2-3 hours. Thereafter they were washed twice with sterile distilled water and cut across

vertically into two halves with sterilised knives under aseptic conditions. A small fraction of the material scraped from the skin-pulp and seed of every fruit was implanted directly in plates on Sabouraud's dextrose agar with antibiotics and left under laboratory conditions for growth. The yeasts thus encountered growing were later obtained in pure form for their final identification.

All the yeast strains isolated were identified up to their specific order by studying their morphological and physiological properties following the standard methods of Lodder & Kreger-van Rij (1952). The results were obtained from one series of experiments with all the fruits except the ones marked with plus signs in Table I, which were planted in two series of plates on two different ocasion.

Results:

The different yeast fungi obtained from the three layers of the fruits are presented in Table I.

The results indicate the presence of yeast fungi in all the three layers of va-

rying depth in the fruits. Of these layers the maximun number was found to occur in the outermost (skin) layer gradually descreasing as we go into the deeper layers. Among the six genera isolated viz. Candida, Cryptococcus, Geotrichum, Rhodotorula, Trichosporon and Torulopsis, the frequency was maximum for the genus Candida which was also represented by maximum number of divergent species.

The results were further analysed in detail to find the frequency of the different species of yeast forms in the three layers of the fruits studied separately. The results presented in Table II clearly indicate that among all the isolates the most common was Candida guilliermondii being closely followed by Candida parapsilosis, the rest being present in very insignificant percentage to note attention, except Trichosporon cutaneum which showed a constant percentage in all the three layers, the outermost layer (skin) presenting the maximum divergent number and variety of yeast fungi inspite of the thorough washing for removing adherent external material. An interesting point to note was the presence of higher number and wider variety of yeasts in the

innermost layer (seed), as compared to the pulp, which naturally is best protected zone of the fruit from the external conditions. (Table II).

Discussion:

With the epoch making discoveries of Penicillin, Streptomycin and other wide range antibiotics the bacterial diseases have lost their unilateral importance, thereby bringing certain mycotic diseases in the forefront for consideration by medical mycologists from public health aspects. Among the various systemic mycotic diseases those caused by yeast fungi have somewhat special place because of the occurrence of some of these fungi even in the normal flora of men and animals. Large number of yeasts have been isolated from the human system associated both with symptomatic and asymptomatic conditions. Although the definite pathogenecity of most of these species of yeasts still await experimental proof, nevertheles it is by far an accepted hypothesis that the yeast flora of men and animals have certain relation and importance with the various pathological condi-

tions met with by various medical mycologists in their study of human disorder. The series of studies conducted and in progress in this laboratory (Bat. et al. loc. cit., Silveira loc. cit., Silveira & Correia loc. cit.) dealing with the presence of yeasts related to symptomatic and asymptomatic conditions in men have established beyond doubt their importance in consideration to human diseases and their public health problems.

An intensive search in the last decade has brought forward with definite proof the fact that soil acts as a natural reservoir for almost all of the human pathogenic fungi (Emmons 1949; 1951; Ajello 1953; 1956; Vanbreuseghem 1952; 1961; Shome 1961; Laurie & Borok 1955; Batista, et al, 1961; Batista et al 1961 a, Shome & Batista, 1961 and others).

A large program that is being carried in the Amazon basin by IMUR x INPA for the forest soil microbiological prospections has showed the presence of yeast fungi as deep in the soil profile as 60 cm. Candida albicans was however isolated from soil samples collected at a depth of 20 cm. from the soil surface (Batista et al 1961, b.).

The presence of yeasts associated with various fruits has been studied for long. As early as 1938 Verona & Ciferri reported Mycotorula albicans associated with a disease in carrots and Baker & Msak in the same year described various strains related with the sugaring of dried figs and prunes. From time to time various reports have appeared dealing with yeast ocouring on fruits and their products. In 1948 Ciferri & Verona in their communication dealing with certain fruits reported in a systematic manner the presence of Rhodotorula, Candida, Torulopsis, Schizosaccharomyces, Kloeckera, Hansenula, Kloeckeraspora and other genera with their divergent species on grape musts and wine. Ciferri, Verona & Luparini (1948) described the isolation of 138 yeast strains from olive oil which appear to have relation with the fruit itself. Sebastiana Joly (1954) in her extensive studies with yeast from large number of fruits of 14 different genera, at São Paulo, was able to isolate 67 pure culture belonging to 4 genera, i.e. Candida, Pichia, Kloeckera and Torulopsis. The simple zymatic species in her studies outweighed the oxydase zymatic ones at a rate of 98,55%. She encoun-

tered many apiculate species presenting low fermentation rates except for glucose. Verona & Toledo (1957) working with grape must of Brazil isolated certain strains of Endomycopsis and studying their morphological and physiological standpoint established a new variety E.fibuliger var. energica.

A critical analysis of the aforesaid results show that they been dealt mainly with the occurrence of yeasts in diseased and healthy fruits and their taxonomical position. No consideration has so far been given to their presence in fruits from the standpoint of its acting as a potencial source for contacting infection when used as food material.

In our opinion the present results throw light on the fact that besides the soil, the regular edible food may as well prove to be a potential reservoir of human pathogenic fungi. Almost all the species of yeast isolated from the various layers of the fruits are well known to be associated with human diseases both superficial and systemic. The species guilliermondii and parapsilosis of the genus Candida have been observed by us (Batista and collaborators) to be

commonly associated with various human disorders. These results although obtained from very limited field of observations, bring to notice, potential danger of a reservoir for these fungi directly and closely related to men being a part of the food and furthermore open a new field to obtain informations for combating of mycotic diseases.

Undoubtedly the present results clearly indicate that the fruits play an important part in the epidemiology of human and animal levedurosis, both when eaten directly in its natural stage and when used as natural juice, salad, icecream etc. The incidence of yeast fungi in human feces in so high a percentage as 76,8%, Batista et al (1961c) may be related with the use of fruits as food. However the role of the animals, domestic and wild, for the spread on soil of yeast fungi through their excreta, is not out of consideration. Practically all of the fruits studied are eaten by these animals, and many of them live in association with human beings and some even as their pets. The yeasts in the fruits may be introduced in the human organism even when used without skin, and in this connection it may be stated that many

persons have the habit to chew the seeds after eating the pulp.

Notwithstanding the presence of yeast fungi in the pulp and seed of the fruits it is recommendable to use the fruits for food after a thorough and careful washing as a preliminary measure for public health hygiene.

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S U M Á R I O

A presente comunicação informa sôbre os resultados da pesquisa efetuada na cidade de Manaus, Amazonas, para o estudo de fungos leveduriformes sôbre frutos comestíveis e silvestres.

Usamos 66 diferentes espécies de frutos, algumas delas espregadas, por mais de uma vez, nos trabalhos de isolamentos de leveduras.

Após lavagem dêsses frutos, por 2-3 horas, seguida de lavagens em água distilada, estéril, foram tomados fragmentos de casca, de polpa e de tegumento de sementes, para implantação em Sabouraud-dextrose-agar.

Os fungos obtidos, os estudamos segundo as técnicas de Lodder & Kreger van-Rij. Os resultados constantes das tabelas apresentadas revelam a presença de leveduras tanto na casca quanto na polpa e nas sementes. Foram identificados fungos dos gêneros Candida, Cryptococcus, Geotrichum, Rhodotorula, Trichosporon e Torulopsis, sendo mais frequentes as espécies do gênero Candida, sobretudo Candida guilliermondii. Trichosporon cutaneum mostrou-se, também, frequente, quer

sôbre a casca quer sôbre a polpa e o tegumento das sementes dos frutos em causa. Uma série de considerações é feita do ponto de vista do assinalamento dos fungos leveduriformes na economia humana, salientando-se, por fim, que os frutos usados na elimentação podem funcionar como vectores na instalação das leveduras humanas e animais, constituindo-se reservatório potencial dêsses microorganismos. Isso faz com que se reforcem as recomendações de emprêgo alimentar dos frutos sòmente após intensa e cuidadosa lavagem, no interêsse da saúde pública, ainda que a polpa e as sementes possam, também, conter fungos.

ZUSAMMENFASSUNG

Diese Arbeit berichtet ueber die Ergebnisse der Untersuchungen, die in der Stadt Manaus im Staat Amazonas, gemacht wurden zum Studium von hefeartigen Pilzen auf essbaren und wilden Fruechten.

Bei der Arbeit zur Isolierung von Hefepilzen wurden 66 verschiedene Arten von Fruechten benuetzt, einige derselben mehr als einmal. Nach Waschung dieser Fruechte waehrend 2-3 Stunden, gefolgt von Waschungen in destilliertem und steriliesiertem Wasser, wurden Fragmente aus den Schalen, aus dem Fleisch und aus den Huellen der Samen entnommen zur Einpflanzung in Sabouraud-Dextrose -agar. Die erlangten Pilze wurden nach den technischen Methoden von Lodder & Kreger-van Rij studiert.

Die erzielten Ergebnisse laut den beiliegenden Tabellen, offenbaren das Vorhandensein von Hefepilzn sowohl in den Schalen, als auch im Fleisch und in den Samen. Es wurden Pilze der folgenden Gattungen identifiziert: Candida, Cryptococcus, Geotrichum, Rhodotorula, Trichosporon und Torulopsis, wobei die folgenden Gattungen die haeufigsten

waren: Candida, hauptsaechlich C. guilliermondii. Trichosporon cutaneum war auch haeufig, sei es auf der Schale, auf dem Mark und auf der Huelle der Samen, der betreffenden Fruechte.

Es wird eine Reihe von Betrachtungen gemacht vom Standpunkt der Bedeutung der hefeartigen Pilze in der Wirtschaft. Und schliesslich hervorgehoben, dass die zur Nahrung gebrauchten Fruechte als Traeger fuer das eindringen der menschlichen und tierischen Hefepilze taetig sein und einen potentiellen Speicher fuer diese Mikroorganismen bilden koennen. Daher wird nochmals empfohlen, dass Nahrungsfruechte, im Interesse der Volksgesundheit, nur nach gruendlicher und sorfaeltiger Waschung zu geniessen sind, obgleich auch das Mark und der Samen Pilze enthalten koennen.

IMUR, 20.10.1961

TABLE I

YEASTS ISOLATED FROM THE SKIN, PULP & SEED OF SIXTY SIX VARIETIES OF FRUITS.

FRUITS	SKIN	PULP	SEED
+1. Castanha do Pará (Bertholletia excelsa)	C. guilliermondii C. parapsilosis	C. guilliermondii C. guilliermondii	Torulopsis candida C. guilliermondii
2. Cupuaçu (Theobroma grandiflorum)	C. guilliermondii	Negative	Trichosporon cutaneum
+3. Babaçu sp. (Orbygnia speciosa)	C. guilliermondii Negative	C. guilliermondii Negative	Torulopsis datilla C. guilliermondii
+4. Umari-roxo (Geoffraea superba)	C. guilliermondii T. famata	C. guilliermondii Negative	C. guilliermondii C. parapsilosis
+5. Umari-amarelo (Geoffraea superba)	T. cutaneum G. candidum	Negative Negative	Negative Trichosporon cutaneum
6. Umari-vermelho (Geoffraea superba)	T. candida	Negative	Geotrichum candidum
+7. Castanha de galinha (Couepia longipendula)	R. mucilaginosa T. famata	Negative	Torulopsis candida
8. Muirá-piranga (Brosimum angustifolium)	T. candida	C. parapsilosis	C. parapsilosis
+9. Piquiá-marfim (Aspidosperma spruceanum)	Negative T. candida	T. cutaneum Negative	C. guilliermondii Torulopsis candida

TABLE I

(contd.)

FRUITS	SKIN	PULP	SEED
10. Muriá-giboia (*Brosimum* sp.)	Negative	Negative	Negative
11. Cardeiro (*Scleronema micranthum*)	Negative	*C. guilliermondii*	Negative
12. Pajurá de racha (*Lucuma speciosa*)	*T. cutaneum*	*C. parapsilosis*	Negative
13. Massaranduba (*Mimusops elata*)	*C. parapsilosis*	*C. guilliermondii*	*C. parapsilosis*
14. Jurubeba (*Solanum* sp.)	*C. parapsilosis*	*C. guilliermondii*	*C. guilliermondii*
15. Sorva (*Couma macrocarpa*)	*C. intermedia*	*C. guilliermondii*	Negative
16. Breu-preto (*Protium* sp.)	*C. parapsilosis*	*C. parapsilosis*	Negative
17. Breu-Almesque (*Protium* sp.)	Negative	Negative	Negative
18. Mairi-bravo (*Geoffraea superba*)	*T. cutaneum*	Negative	Negative
+19. Abiorana (*Lucuma dissepala*)	Negative *C. parapsilosis*	Negative Negative	Negative Negative

TABLE I

(contd.)

FRUITS	SKIN	PULP	SEED
+20. Marajá (*Bactris marajá*)	*C. tropicalis* *T. candida*	*C. guilliermondii* Negative	*C. guilliermondii* *C. parapsilosis*
21. Louro-gamela (*Ocotea sp.*)	*Trichosporon cutaneum*	*C. parapsilosis*	*C. parapsilosis*
+22. Pachiubinha	*C. guilliermondii* *Candida krusei*	*C. parapsilosis*	Negative
23. Paratudo (*Simaba sp.*)	Negative	*C. parapsilosis*	*C. guilliermondii*
24. Pepino-bravo (*Luffa sp.*)	*C. guilliermondii*	Negative	Negative
25. Pupunha (*Guilielma speciosa*)	*C. parapsilosis*	*C. parapsilosis*	*C. parapsilosis*
26. Biribá (*Rollinia mucosa*)	*C. parapsilosis*	Negative	*C. parapsilosis*
+27. Tucumã (*Astrocaryum sp.*)	*C. guilliermondii* *C. guilliermondii*	*C. guilliermondii* Negative	*C. parapsilosis* *C. guilliermondii*
28. Batatão (tubérculo)	*C. tropicalis*	Negative	
29. Cacau (*Theobroma cacao*)	*C. guilliermondii*	Negative	Negative

T A B L E I

(contd.)

FRUITS	SKIN	PULP	SEED
30. Pupunha-brava (*Duckeodendron sp.*)	*Cryptococcus laurentii*	Negative	*Candida parapsilosis*
31. Bacuri-bravo (*Moronobea candida*)	Negative	Negative	Negative
32. Andiroba (*Carapa guyanensis*)	*Candida guilliermondii*	Negative	Negative
33. *Licania sp.*	*Candida tropicalis*	*Candida parapsilosis*	Negative
34. Cajá (*Spondias lutea*)	*Candida guilliermondii*	*Candida parapsilosis*	*Candida parapsilosis*
35 Cupui (*Thebroma subincanum*)	Negative	*Trichosp cutaneum*	*Torulopsis famata*
36. Envireira (*Sterculia sp.*)	*Candida guilliermondii*	*Trichosp cutaneum*	*Candida guilliermondii*
37. Goiaba (*Psidium guajava*)	Negative	*Candida parapsilosis*	Negative

TABLE I

(contd.)

FRUITS	SKIN	PULP	SEED
38. Murici (Byrsonima crassifolia)	Candida guilliermondii	Candida parapsilosis	Trichosporon cutaneum
39 Swartzia sp	Negative	Negative	Negative
40. Cajarana (Spondias cytherea)	Negative	Negative	Candida guilliermondii
41. Atumã	Negative	Negative	Negative
42. Mari-mari (Poraqueiba sp.)	Negative	Negative	Geotrichum candidum
43. Maracujá de capoeira (Passiflora sp.)	Negative Negative	Negative Negative	Negative Negative
44. Cacau do mato (Theobroma cacao)	Negative	Negative	Negative
45. Genipapo do igapó (Genipa americana sp.)	Negative	Negative	Negative
46. Uchi (Saccoglottis uchi)	Candida guilliermondii	Candida guilliermondii	Negative

T A B L E I

(contd.)

FRUITS	SKIN	PULP	SEED
47. Tucumai (*Bactris sp.*)	Negative	Negative	Negative
48. Uchi-amarelo (*Saccoglottis uchi*)	*Candida parapsilosis*	Negative	*T. cutaneum*
49. Caranai	Negative	Negative	Negative
50. Murupá	Negative	Negative	*C. parapsilosis*
51. Buriti (*Mauritia vinifera*)	Negative	Negative	Negative
52. Buiuçu (*Ormosia coutinhoi*)	*Trichosporon cutaneum*	*C. guilliermondii*	Negative
53. Ubaia (*Eugenia uvalha*)	*C. guilliermondii*	*T. cutaneum*	*C. guilliermondii*
54. Apui-grande (*Ficus sp.*)	Negative	Negative	Negative
55. Uchi-coroa (*Saccoglottis uchi*)	*Geotrichum candidum*	Negative	*C. parapsilosis*
56. Jurubeba-branca (*Solanum sp.*)	*C. guilliermondii*	*C. guilliermondii*	*C. guilliermondii*

T A B L E I

(contd.)

FRUITS	SKIN	PULP	SEED
57. Grão de galo (Tabernaemontana laeta)	Candida intermedia	C. guilliermondii	Candida guilliermondii
58. Sorvea-pequena (Couma utilis)	Negative	Negative	Negative
59. Ata (Anona muricata)	Candida tropicalis	Candida tropicalis	Candida tropicalis
60. Catolé (Syagrus oleracea)	Candida albicans	Candida albicans	Candida albicans
61. Goiaba de anta (Picramnia sellowii)	C. guilliermondii	T. cutaneum	Trichosporon cutaneum
62. Ingai (Inga sp.)	Negative	Negative	Negative
+63. Jurubeba de rama (Solanum sp.)	Candida tropicalis Candida parapsilosis	Negative	Negative
64. Rosaceae sp.	Negative	Negative	Negative
65. Ingá-chinelo (Inga sp.)	Negative	Negative	Negative
66. Menispermaceae sp.	Negative	Negative	Negative

TABLE II

FREQUENCY OF YEAST FUNGI IN DIFFERENT LAYERS OF 66 FRUITS STUDIED

FRUITS	SKIN	PULP	SEED
1. *Candida guilliermondii*	17 (25.70%)	14 (21.21%)	13 (19.69%)
2. *Candida albicans*	1 (01.51%)	1 (01.51%)	1 (01.51%)
3. *Candida parapsilosis*	9 (13.60%)	11 (16.66%)	12 (18.18%)
4. *Candida intermedia*	2 (03.03%)	-	-
5. *Candida krusei*	1 (01.51%)	-	-
6. *Candida tropicalis*	5 (07.57%)	1 (01.51%)	1 (01.51%)
7. *Cryptococcus laurentii*	1 (01.51%)	-	-
8. *Geotrichum candidum*	2 (03.03%)	-	2 (03.03%)
9. *Rhodotorula mucilaginosa*	1 (01.51%)	-	-
10. *Trichosporon cutaneum*	5 (07.57%)	5 (07.57%)	5 (07.57%)
11. *Torulopsis candida*	4 (06.06%)	-	2 (03.03%)
12. *Torulopsis famata*	2 (03.03%)	-	1 (01.51%)
13. *Torulopsis dattila*	-	-	1 (01.51%)